

Comparison of throat swabs with sputum specimens for the detection of *Chlamydia pneumoniae* antigen by direct immunofluorescence

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Abstract

Aim—To compare throat swabs with sputum specimens for *Chlamydia pneumoniae* antigen detection.

Methods—During a one year period, sputum and throat swabs from 50 patients over 15 years of age with acute or persisting lower respiratory tract infection were examined for *C pneumoniae* antigen by direct immunofluorescence.

Results—*C pneumoniae* antigen was detected in 18/50 patients (36.0%) from sputum, throat swab, or both. Paired sputum and throat swabs were received from 35/50 patients (70.0%). *C pneumoniae* antigen was detected in either or both specimens from 14/35 patients (40.0%). Of the 14 positive patients, both specimens were positive in nine (64.3%), throat swab only in four (28.6%), and sputum only in one (7.1%). Of the remaining 15 patients from whom only a single specimen was sent, a further three of eight throat swabs and one of seven sputum specimens were positive. There was no statistically significant difference between the results obtained from the two types of specimen.

Conclusions—Throat swabs may be as good as sputum for the detection of *C pneumoniae* antigen.

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Keywords: *Chlamydia pneumoniae*; throat swabs; direct immunofluorescence

Chlamydia pneumoniae is a common respiratory pathogen which causes about 10% of community acquired pneumonias.¹ The organism is of special interest because of the suggested relation between persistent infection and adult onset asthma² and recurrent infection and coronary heart disease,^{1,3} but the best method of microbiological diagnosis at the acute stage of infection is undecided. Serology is retrospective, the organism grows poorly in cell culture,¹ and polymerase chain reaction (PCR) tests⁴ are not generally available. Antigen detection in sputum specimens by direct immunofluorescence (DIF) confirmed enzyme linked immunosorbent assay (ELISA) is feasible at the acute stage of infection,⁵ and is a suitable method for the routine laboratory.

We compared throat swabs with sputum specimens for the detection of *C pneumoniae* by DIF alone. The purpose of the study was to decide which type of specimen was most suitable for diagnosis of *C pneumoniae* by DIF.

Methods

SELECTION OF PATIENTS

Consultant chest physicians were asked to submit specimens for *C pneumoniae* examination from hospital inpatients over 15 years of age with lower respiratory tract infection, bronchitis, exacerbation of chronic obstructive airways disease, or persisting chest infection between 1 November 1991 and 31 October 1992.

NUMBER OF PATIENTS

The study was continued prospectively until 50 such patients had been recruited.

SPECIMENS

A sputum specimen and throat swab were requested from all patients during the acute stage of illness. Sputum specimens were examined by naked eye and specimens which were mainly saliva or mucus were rejected. Throat swab specimens were accepted only if numerous squamous epithelial cells were seen on DIF microscopy.

DIF METHOD FOR SPUTUM AND THROAT SWABS

Throat swabs were collected into chlamydia transport medium (Northumbria Biologicals, Cramlington, Northumbria, UK). This transport medium was chosen because experience of its use for DIF diagnosis of *C trachomatis* showed that comparable results were obtainable by DIF, ELISA, and culture.⁶ Throat swabs were vortex mixed in the transport medium on receipt, and 0.5 ml of the suspension centrifuged in an MSE microcentaur centrifuge at 11 600 g for 10 minutes. The supernatant was discarded and the pellet resuspended in 1 ml of distilled water. The procedure of vortexing and centrifugation was repeated once, and the supernatant discarded to leave approximately 50–100 µl, which was again vortexed to yield a milky suspension. A 5 µl amount was placed on a single well of a 15 well teflon coated slide (Multispot Microscope Slides, CA Hendley (Essex), UK), dried and fixed in methanol for five minutes, drained, and air dried before staining. Sputum was digested with an equal volume of freshly prepared 2% N-acetyl-cysteine (Sigma, Poole, Dorset, UK), for 10–30 minutes using a mechanical shaker at 75–90 oscillations/min, centrifuged at 11 600 g for 10 minutes, double washed in distilled water by vortexing and centrifugation, and then processed in the same way as the throat swabs.

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Table 1 Detection of *Chlamydia pneumoniae* antigen by direct immunofluorescence in throat swabs and sputum from 50 patients

Specimens received	Number of patients	Antigen detection by microimmunofluorescence			
		Sputum +	Sputum -	Throat swab +	Throat swab -
Throat swab + sputum	35	10	25	13	22
Sputum only	7	1	6		
Throat swab only	8			3	5
Total result		11	31	16	27
Total No of specimens		42		43	

Staining

The Chlamydia-Cel Pneumoniae IF kit (Cel-labs Diagnostics, Brookvale, New South Wales, Australia; TCS Microbiology, Botolph Claydon, Buckingham, UK) was used. This kit employs a type specific monoclonal IgM antibody for *C pneumoniae* detection. The antigen detected is the outer membrane protein. The manufacturers specify that the monoclonal antibody has been extensively checked for cross reaction with other *Chlamydia* species and found to be negative. A 5 µl amount of *C pneumoniae* monoclonal antibody supplied with the kit was added to each specimen well. Slides were incubated in a moist chamber at 37°C for 30 minutes, then washed in phosphate buffered saline (PBS), pH 7.4, for approximately one minute. Each well received 5 µl antimouse Ig-FITC reagent supplied with the kit. The slide was incubated in a moist chamber in the dark at 37°C, washed in PBS, and a drop of mounting fluid added to the slide before applying a coverslip and examining by fluorescence microscopy with an oil immersion lens at ×600 and ×1000 magnification.

Slide reading

The control slide supplied with the monoclonal antibody kit was used to check the morphology of the elementary bodies. A test was deemed positive when four or more elementary bodies, appearing as bright apple-green fluorescent disc shaped bodies about 300 nm in diameter, were seen. Positive and negative control slides were included in each test. All slides were initially read by the same person, then all were independently checked by another member of staff experienced in the diagnosis of *C trachomatis* by DIF. In addition, all *C pneumoniae* DIF positive slides were repeated and rechecked by the two observers to ensure that all positive results were reproducible.

STATISTICAL METHODS

Confidence intervals were applied to *C pneumoniae* prevalence results. Results of throat swab and sputum specimens were compared using a Yates corrected χ^2 formula.

Results

The specimens received and the results of *C pneumoniae* antigen detection are shown in table 1. All positive results were confirmed on retesting.

Of the 35 patients from whom paired sputum and throat swab specimens were received, 14 were positive for *C pneumoniae*. Both sputum and throat swab were positive in nine (25.7%), both were negative in 21

(60.0%), sputum positive but throat swab negative in one (2.9%), and sputum negative but throat swab positive in four (11.4%).

Antigen was detected in 18/50 patients (36.0%) in sputum, throat swab, or both (95% confidence interval 0.24 to 0.47).

There was no statistically significant difference in the number of *C pneumoniae* positive results detected by throat swabs and sputum specimens ($p > 0.01$).

Discussion

No method for the detection of *C pneumoniae* by the routine microbiology laboratory is ideal. Culture is difficult and is usually done from throat swabs, as there is no satisfactory method for sputum culture.¹ Micro-IF serology is specific and sensitive, but its clinical value is limited, because it takes three to eight weeks for antibodies to appear and its interpretation can be difficult without a knowledge of local seroprevalence, especially as antibody patterns may be complex.¹

The alternative is to attempt direct detection from respiratory specimens. Sputum antigen detection by PCR,⁴ or DIF confirmed ELISA,⁵ have both been used⁵ and are suitable for diagnosis at the acute stage of infection. PCR is a sensitive and specific method for sputum, which gives good concordance with DIF antigen detection,⁴ but it is a specialised method. ELISA is only marginally less sensitive than DIF for the detection of chlamydia antigen in sputum,⁵ and is a suitable test for the routine diagnostic laboratory, especially if it can be confirmed by DIF speciation. The problems are the difficulty of obtaining sputum from some patients, and of attaining the right degree of sputum homogenisation to release chlamydial antigen while avoiding the false positive results which can be caused by incomplete digestion.³ The possibility of false positive results caused by incomplete digestion is difficult to solve. We found no statistical difference between results obtained from throat swabs (not treated by digestion) and sputum specimens (treated by digestion). This suggests that false positive sputum results caused by incomplete digestion are perhaps relatively infrequent. For the same reason, it seems unlikely that the transport medium used had any adverse effect, since only the throat swabs were sent in transport medium.

The *C pneumoniae* prevalence of 36% found in the miscellaneous group of chest medicine patients from whom specimens were submitted was unexpectedly high. The possibility of a local epidemic at the time of the study cannot be excluded, but no definite conclusion can be made because of the small number of patients studied and the highly selected group of respiratory medicine patients from which they were drawn. This is reflected in the wide 95% confidence interval range. In addition, serological studies suggest that *C pneumoniae* may persist for months after acute infection.⁷ As we made no attempt to differentiate between acute infection and persistent carriage, it was impossible to say whether patients had an acute infection or not.

We found that there was no statistical difference between the results given by sputum and throat swab specimens for DIF *C pneumoniae* detection. The advantage of throat swabs is that they are easy to obtain and process and avoid the technical problems of sputum homogenisation⁵ and the practical difficulty of obtaining sputum. Throat swabs may be a useful and more convenient alternative to sputum specimens for *C pneumoniae* DIF diagnosis.

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